

ARIC Manuscript Proposal # 3197

PC Reviewed: 7/10/18
SC Reviewed: _____

Status: _____
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Priority: 2
Priority: _____

1.a. Full Title:

Plasma cyclic guanosine monophosphate (cGMP) and incident atherosclerotic cardiovascular disease and heart failure: the Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters):

Cyclic GMP and ASCVD/HF

2. Writing Group:

Writing group members:

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I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. DZ **[please confirm with your initials electronically or in writing]**

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3. Timeline:

The manuscript will be complete within 2-3 months upon the approval of this proposal.

4. Rationale:

Despite the declining mortality, atherosclerosis cardiovascular disease (ASCVD), including coronary heart disease (CHD) and stroke, remains the leading cause of death in the US.¹ Furthermore, heart failure (HF) incidence is on the rise with 6.5 million U.S. adults living with HF in 2014.¹ These statistics underscore the need to identify novel risk factors and treatment strategies for cardiovascular disease.

Cyclic guanosine monophosphate (cGMP) is an intracellular second messenger of a wide array of signaling functions, and it mediates many physiological processes in the cardiovascular system.²⁻⁵ The cGMP signaling cascade has been implicated in ASCVD, HF, and cardiac dysfunction. Recent studies suggested that exploring steps of cGMP pathways, from cGMP synthesis to inactivation, could provide novel pharmaceutical tools to improve outcomes in ASCVD or HF patients.² Indeed, agents such as stimulators and activators of cGMP, have been shown to be potential candidates in treating various heart conditions, including HF with preserved ejection fraction (HFpEF),⁶ acute decompensated HF,⁷ and pulmonary arterial hypertension.⁸

The mechanisms of cGMP regulation have not been fully understood. Bench research and animal models suggested that cGMP can activate two effector molecules: cGMP-dependent protein kinase (PKG) and phosphodiesterases (PDEs). Additionally, cGMP is synthesized through two different reciprocal pathways with different downstream effects: the nitric oxide/soluble guanylyl cyclase GC (NO/sGC) pathway and the natriuretic peptide/guanylate cyclases-A (NP/GC-A) pathway. The NO/sGC pathway exerts its effect by suppressing cardiomyocyte and left ventricular hypertrophy, decreasing contractility, relaxing endothelial and vascular smooth muscle cells, inhibiting ischemia-reperfusion injury, and countering maladaptive remodeling.^{2-5, 9} The NP/GC-A pathway is stimulated in conditions of cardiac overload, and acts to unload the heart by natriuresis, diuresis, vasodilation, renin- and aldosterone suppression.¹⁰ The two pathways can have different distribution in intracellular receptors. Compared with NO/sGC pathway, NP/GC-A stimulation can result in increases in distinct cGMP pools that are compartmentalized by PDE activity.¹⁰

Research on cGMP has mainly been done using ex-vivo and animal models and population-based studies on cGMP are limited. In a survey study conducted among Japanese men and women, urinary cGMP was inversely associated with total and non-HDL cholesterol.¹¹ Another study among patients with obstructive sleep apnea found that urinary cGMP was markedly reduced in hypertensive compared to non-hypertensive patients.¹² However, the role of cGMP in development of ASCVD or HF events has never been assessed in a general community-based ethnically diverse cohort.

We recently measured plasma cGMP in a subset of women and men in the ARIC cohort (at visit 4) as part of an ancillary study funded by an American Heart Association Go Red for Women Strategic Focused Research Network grant. This proposal will study the association between cGMP and ASCVD and HF outcomes in the prospective, large scale, bi-ethnic, community-based cohort, which is fundamental in providing insight that may point to potential novel prevention and treatment regimens for ASCVD and for HF.

5. Main Hypothesis/Study Questions:

We hypothesize that the cGMP cascade plays an important role in mediating ASCVD- and HF- related events, and the associations differ by sex. Specifically, we hypothesize that higher cGMP levels will be associated with higher risk of total ASCVD, CHD, stroke, HF, and its subtype HFpEF in women but not men.

6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).

Study Design:

This study will use data from 1,034 ARIC participants selected by HFpEF case-cohort design with cGMP measured using blood samples from visit 4.

The selection of the ARIC V4 participants for inclusion in the case-cohort analysis in AHA Go Red for Women (GRFW) project was as follows:

- Number of ARIC participants attending V4: 11,656.
- Number of ARIC V4 participants excluded from AHA GRFW project: 1,950.
 - Prevalent heart failure at ARIC V1: 412.
 - HF hospitalization prior to 1/1/2005: 865.
 - Died before 1/1/2005: 1,067.
- Number of ARIC V4 participants eligible for AHA GRFW project: 9,706.
 - Number included in Random Control Subcohort: 700 (fixed by design).
 - Number not included in Random Control Subcohort: 9,006.
- Number of incident HFpEF cases among eligible participants between 1/1/2005 and 12/31/2013: 332.
 - Number of incident HFpEF cases among Random Control Subcohort: 25.
- Number of participants selected for analysis in the case-cohort design: 1,007 (700 participants in the Random Control Subcohort + 332 incident HFpEF cases – 25 overlap participants).

cGMP measurement

Cyclic GMP levels were measured from plasma samples collected at visit 4 that were stored at -80° until their measurement in 2017. cGMP concentrations were assessed using a competitive ELISA assay (Cayman Chemical Company, MI) at the Baylor University Laboratory, with the addition of an optional acetylation procedure per manufacturer protocol. Intra and inter-assay coefficients of variation for a control pool with a mean cGMP value of 6.5 pmol/mL were 4.2% and 13.5%, respectively.

Exclusion:

- Not of either Black or White race
- Blacks from Minnesota and Maryland center
- Prevalent CVD or HF prior to visit 4

Primary endpoints:

- Incidence of heart failure
- Incidence of heart failure with preserved ejection fraction (HFpEF)
- Incidence of cardiovascular disease.
- Incidence of coronary heart disease.
- Incidence of stroke

Measurement of endpoints

Incident cardiovascular events in ARIC were ascertained by annual telephone contact and surveillance of hospital and death records. CHD was defined as definite or probable myocardial infarction, definite CHD death or coronary revascularization. ASCVD was defined as CHD plus definite or probable stroke (defined as sudden or rapid onset of neurologic symptoms that lasted for 25 hours or led to death in the absence of another cause). Heart failure was defined as the first HF hospitalization (ICD-9 code 428 in any position), or any deaths where the death certificate included an HF code (code 428, ICD-9 or 150, ICD-10, in any position). HFpEF was defined as incident acute decompensated HFpEF or ARIC adjudicated chronic stable HF and (current and previous ejection fractions both $\geq 50\%$ or one of ejection fractions $\geq 50\%$ and the other one missing)

Statistical analysis

The baseline for this study is visit 4, the time of the cGMP measurement. We will examine the distribution of cGMP by age, sex, and race. To evaluate the association between cGMP levels and incidence of CVD outcomes occurring between visit 4 through December 31, 2016, we will use Cox proportional hazard models with sampling weights for the case-cohort design to account for the over-sampling of participants with HFpEF and provide estimates applicable to the overall ARIC population.

cGMP will be modeled continuously and in tertiles. In addition, we will model cGMP using restricted cubic splines (with knots at the 5th, 35th, 65th, and 95th percentiles of its sample distribution) to provide a flexible assessment of the dose-response relationship between cGMP and incident ASCVD/CHD/HF risk. All covariates will be from visit 4 (except for education, which was assessed at visit 1). The models will be adjusted as follows:

- Model 1: Adjusts for age, sex, race/center groups
- Model 2: Includes model 1, plus education, smoking, alcohol consumption, BMI, and physical activity.
- Model 3: Includes model 2 plus diabetes, systolic blood pressure, use of anti-hypertensive medications, total cholesterol, HDL cholesterol, use of lipid-lowering therapy, and eGFR
- Model 4: Includes model 3 plus log-transformed NT-pro-BNP*
- Model 5: Includes model 3 plus estradiol**

*As mentioned in the introduction, the natriuretic peptides (i.e. NT-pro-BNP) are upstream of cGMP pathway.

**Estradiol is upstream of the NO-cGMP-PKG pathway.

In a separate analysis, we will also evaluate the association between cGMP /NT-pro-BNP ratio and the ASCVD/HF endpoints. Interaction analyses will be explored in pre-specified subgroups defined by age, sex, and race. If an interaction is found, results will be stratified.

7.a. Will the data be used for non-CVD analysis in this manuscript? Yes
 No

b. If Yes, is the author aware that the file ICTDER03 must be used to exclude persons with a value RES_OTH = "CVD Research" for non-DNA analysis, and for DNA analysis RES_DNA = "CVD Research" would be used?

Yes No

(This file ICTDER has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript?
 Yes No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER03 must be used to exclude those with value RES_DNA = "No use/storage DNA"?

Yes No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at:

<http://www.csc.c.unc.edu/ARIC/search.php>

Yes No

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

No prior ARIC manuscript proposals have used information on cyclic GMP.

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? Yes No

11.b. If yes, is the proposal

A. primarily the result of an ancillary study (list number*
2015.21_____)

B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* _____
_____)

*ancillary studies are listed by number at <http://www.csc.c.unc.edu/aric/forms/>

12a. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is **your responsibility to upload manuscripts to PUBMED Central** whenever the journal does not and be in compliance with this policy. Four files about the public access policy from <http://publicaccess.nih.gov/> are posted in <http://www.csc.unc.edu/aric/index.php>, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to Pubmed central.

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